183.9

MUCOSAL ADMINISTRATION OF HSP 65 DECREASES ATHEROSCLEROSIS AND INFLAMMATION IN THE AORTIC ARCH OF LDL RECEPTOR DEFICIENT MICE

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Increasing evidence supports the involvement of inflammation and immunity in atherogenesis, as well as the role of autoimmunity to heat shock proteins in the progression of atherosclerosis. Mucosal administration of autoantigens decreases organ specific inflammation and disease in animal models (diabetes, arthritis and EAE) and is being tested in human clinical trials. We examined the effect of nasal or oral administration of HSP65 on atherosclerotic lesion formation in mice lacking the receptor for low-density lipoprotein maintained on a high cholesterol diet. Animals were nasally treated with 0.8ug HSP 65 three times every second day or orally treated with 8 ug HSP 65 on 5 consecutive days. A high cholesterol diet was started after the last treatment and mice were mucosally treated once/week for 8 weeks at which time pathologic analysis was perfomed. In nasally treated animals, we found a reduction in macrophage-positive area in the aortic arch (3.44% vs. 13.03% in controls, $p = 0.006$) as well as a reduced number of T-cells ($p = 0.02$). There was also a decrease in the size of atherosclerotic plaques. A similar trend was observed in orally treated animals but was not significant. Mice nasally treated with HSP also gained significantly less weight than fed or control treated mice. Our results suggest that nasal treatment with HSP reduces the inflammatory process associated with atherosclerosis and may provide a new treatment approach.

183.11

Phase I Clinical Trial of Orally Delivered Hepatitis B Surface Antigen Expressed in Potato Tubers.

[1]Yasmin Thanavala, [1]Adrienne Scott, [1]Srabani Pal, [1]Martin Mahoney and [2]Charles Arntzen. [1]Roswell Park Cancer Institute, Buffalo, NY; [2]Boyce Thompson Institute for Plant Research, Ithaca, NY.

A randomized, doubleblind, placebo-controlled phase I clinical trial has been completed at Roswell Park Cancer Institute to evaluate the safety, tolerability and immunogenicity of orally delivered HBsAg expressed as a protein in transgenic potatoes. Forty-five healthy healthcare workers with a history of known positive responses

183.7

CHOLERA TOXIN B SUBUNIT AS MUCOSAL CARRIER-DELIVERY SYSTEM FOR SPECIFIC IMMUNOTHERAPY.

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Over the past few years attention has been devoted to the development of effective formulations that could prevent or halt untoward immune responses, such as those underlying autoimmune disorders, allergic reactions, and by and large chronic inflammation. Studies initiated in this laboratory have documented the efficiency of cholera B subunit as a powerful mucosal immunomodulating and carrier-delivery system agent for optimal induction of immune tolerance in various preclinical models of autoimmune diseases. More recently, this ystem has proven to be especially effective for suppressing type I allergic responses and also for suppressing Th2-driven immunopathological responses to persistent infectious microorganisms. The mechanisms of action of this system and in particular the role of mucosal dendritic cells in the induction of such form of suppression is currently under study. These studies will be presented and their implications will be discussed. (supported by INSERM, Swedish Medical Research Council, European Communities EC Biotech IV NovoNordisk, Triotol)

183.8

MYELIN-SPECIFIC TOLERANCE ATTENUATES DISEASE SEVERITY IN A VIRALLY INDUCED MODEL OF MULTIPLE SCLEROSIS.

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Theiler's Murine Encephalomyelitis Virus-induced Demyelinating Disease (TMEV-IDD) is a relevant model for the autoimmune disease multiple sclerosis (MS). Approximately 30 days after intracerebral inoculation of S.J. mice with TMEV, clinical disease signs arise, characterized by spastic paralysis, chronic disease progression, and mononuclear cell infiltrate into the CNS. While initial demyelination in TMEV-IDD is mediated by virus-specific CD4+ T cells, reactivity to myelin epitopes can be detected in TMEV infected mice 55 days post infection, demonstrating autoimmune specificity in this virally induced disease. Administration of the fusion protein MP4, a fusion of myelin proteins MBP and PLP, to TMEV infected SJL mice 40 days post infection attenuates disease severity in MP4 treated animals compared to controls, and also decreases DTH reactivity to myelin peptides, indicating anti-myelin responses are centrally involved in the chronic progressive nature of TMEV-induced paralysis. Additionally, T cells isolated from the spinal cords of TMEV infected animals proliferate and secrete IFNγ in response to PLP139-151 peptide stimulation in vitro. Both isolation of myelin specific cells from the CNS of TMEV infected animals, and myelin specific tolerance in TMEV-IDD indicate anti-myelin T cell responses contribute to disease severity in this virally induced model of MS, and support the idea of antigen specific tolerance as an effective treatment of ongoing autoimmune disease. (Supported by NIH grant NS23349)

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Increasing evidence supports the involvement of inflammation and immunity in atherogenesis, as well as the role of autoimmunity to heat shock proteins in the progression of atherosclerosis. Mucosal administration of autoantigens decreases organ specific inflammation and disease in animal models (diabetes, arthritis and EAE) and is being tested in human clinical trials. We examined the effect of nasal or oral administration of HSP65 on atherosclerotic lesion formation in mice lacking the receptor for low-density lipoprotein maintained on a high cholesterol diet. Animals were nasally treated with 0.8ug HSP 65 three times every second day or orally treated with 8 ug HSP 65 on 5 consecutive days. A high cholesterol diet was started after the last treatment and mice were successfully treated once/week for 8 weeks at which time pathologic analysis was performed. In nasally treated animals, we found a reduction in macrophage-positive area in the aortic arch (3.44% vs. 13.03% in controls, $p = 0.006$) as well as a reduced number of T-cells ($p = 0.02$). There was also a decrease in the size of atherosclerotic plaques. A similar trend was observed in orally treated animals but was not significant. Mice nasally treated with HSP also gained significantly less weight than fed or control treated mice. Our results suggest that nasal treatment with HSP reduces the inflammatory process associated with atherosclerosis and may provide a new treatment approach.

183.10

HIGH DOSE -ANTIGEN FEEDING INDUCES CD4 T CELLS WITH SUPPRESSOR ACTIVITY IN THE LIVER.

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Oral feeding of low or high dose-antigens (Ag) induces Ag-specific immuno-suppression in subsequent systemic challenge with the same Ag. Since a part of Ag fed at high dose should reach to the liver as an immunogenic form, we examined the possibility that Ag-specific T cells are activated by high dose-Ag feeding. OVA-TCR transgenic mice were fed 100 mg or 1mg of OVA, or PBS every other day for five times and then CD4 T cells were purified from Peyer's patch, spleen, and liver. Only intrahepatic CD4 T cells (IHLs) from high dose Ag-fed mice suppressed both Ag-specific DTH and antibody responses when adoptively transferred to naive Balb/c mice. Upon Ag-stimulation in vitro, the secretion of IL-10, TGF-beta, and especially IL-4 by IHLs from Ag-fed mice were increased in an Ag-dose dependent manner. In contrast, IL-2 secretion and proliferative responses by these T cells were decreased. In addition, these IHLs from Ag-fed mice inhibited Ag-specific proliferation of naive splenic CD4 T cells. FACS analysis revealed decrease in the population of Ag-specific CD4 T cells in the liver by Ag-feeding, associated with the up-regulation of Fas-L expression, suggesting that clonal deletion was induced in the liver. Naive splenic CD4 T cells cultured with OVA presented by liver-derived APCs showed a similar profile of cytokine production to that of IHLs. Taken together, these data suggest that high dose-Ag feeding induces CD4 T cells with suppressor activity in the liver. Not only clonal deletion but also active suppression is considered to be induced in the liver after high dose-Ag feeding.

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A randomized, doubleblind, placebo-controlled phase I clinical trial has been completed at Roswell Park Cancer Institute to evaluate the safety, tolerability and immunogenicity of orally delivered HBsAg expressed as a protein in transgenic potatoes. Forty-five healthy healthcare workers with a history of known positive response to a primary series of recombinant hepatitis B vaccine (meeting all inclusion criteria and none of the exclusion criteria) were recruited for the trial. The 45 volunteers were randomized into one of three groups. Each group ate either vaccinated or placebo potato at defined intervals. Study subjects were randomized by use of a centrally generated block randomization list. This list was provided to the study pharmacist who was unblinded to study group assignments. All other study personnel and the study subjects remained blinded through the completion of the study. Subjects had baseline chemistry, hematology and anti-HBs antibody determinations performed before their first dose of vaccine and at predetermined intervals throughout the trial. As a phase I study, this was primarily an assessment of the relative safety and immunogenicity of transgenic HBsAg expressing potatoes.

183.12

ORAL IMMUNIZATION BY FOOD IS LESS EFFECTIVE THAN INTRAGASTRIC IMMUNIZATION

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The feasibility of edible vaccines was studied by oral immunization of mice with chicken ovalbumin (OVA) mixed with standard food. Other mice were immunized with a similar dose of OVA via intragastric immunization. Intragastric immunization elicited 20-fold higher numbers of anti-OVA IgA and 38-fold higher numbers of anti-OVA IgG producing cells in the lamina propria of the gut than food immunization. Furthermore, intragastric immunization elicited a 20-fold higher anti-OVA IgG response in serum and a 2-fold higher anti-OVA IgA response in faeces than food immunization. The addition of the *Vibrio cholerae* toxin to food did not enhance the immune response. Possible explanations for the differences between these immunization routes will be discussed. We concluded that intragastric immunization is merely limited indicative for the effectiveness of edible vaccines.